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Measles Immunity and Response to Revaccination of a Young Adult Population in Israel

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In order to evaluate the true immune status and the effect of revaccination on a young adult population, we collected serum samples from 289 military recruits who were vaccinated during an outbreak in 1991. Most vaccinees, age 18–25 years, had apparently been immunized once before as infants. Sera collected just prior to the vaccination and 14 and 28 days afterwards were tested for measles antibodies by hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA)-IgM.

Before vaccination, 46 (15.9%) of the subjects had no HI antibodies, (<1:4) and 48 (16.6%) had borderline (1:4) HI titer. Following vaccination, only ten (3.5%) remained negative and 19 (6.6%) had borderline titer. The increase in HI antibody titer was inversely proportional to the prevaccination titer, and 159 subjects (55.0%) showed no increase at all. The geometric mean titer (GMT) rose from 9.14 to 21.47. Among the prevaccination-negative subjects (HI <1:4) 28 (60.9%) reached a postvaccination titer of ≥1:8, and eight (17.4%) reached a titer of 1:4. Twelve (26.1%) of the negative subjects seroconverted and developed IgM, 16 (35%) seroconverted without IgM, and 18 (39%) remained negative and did not develop IgM.

A group of eight vaccinees with prevaccination titer of ≥1:4 developed IgM. Some were probably infected by the circulating wild-type virus prior to the vaccination. Thus, a total number of 20 of the 289 subjects studied (6.9%) had true negative preimmune status as judged by the IgM test. However, the vaccination campaign prevented further measles cases, apparently by increasing the population's immunity, particularly in individuals with very low titers or without measles antibodies.

KEY WORDS: HI, IgM, outbreak, immunization

INTRODUCTION

Measles is a highly contagious, widespread disease which has been put under control since 1966, following the use of live attenuated virus vaccine [Black, 1989; Katz and Gellin, 1994; Orenstein et al., 1994].

Vaccine coverage in most of the world exceeds 80% [Katz and Gellin, 1994; Orenstein et al., 1994], but there are still more than 40 million cases and about 1 million deaths annually, most of them in developing countries. The immunization schedule at age 12–15 months (in developed countries) or at age 6–9 months (in developing countries) with introduction of a second dose in infancy or school age in some countries [MMWR, 1989, 1994b; Tulchinsky et al., 1993] did not cause eradication of the disease. Outbreaks still occur in well-vaccinated communities [Atkinson et al., 1991; Gustafson et al., 1987., Orenstein et al., 1980; MMWR, 1994a,b; Orenstein et al., 1994; Tulchinsky et al., 1993; Shasby et al., 1977; Aaby et al. 1990; Edmonson et al., 1990; Murphy et al., 1984]. In developing countries, large outbreaks occur in young babies who have lost their maternal antibodies but have not yet been vaccinated [Aaby et al., 1990; Katz and Gellin, 1994; Orenstein et al., 1994; Tulchinsky et al., 1993; Pabst et al., 1992] and among school-age children as a result of primary vaccine failure and low vaccination coverage. In developed countries most cases appear in clusters in populations of young adults as a result of both primary and secondary vaccine failure [Black, 1989; Orenstein et al., 1994; Kawamoto et al., 1995; MMWR, 1994a; Edmonson et al., 1990; Ammari et al., 1993]. Measles cases also occur in non-immunized populations, including babies prior to their first dose. In the United States following the resurgence of measles in 1990–1991, a second dose was recommended either in the 2nd year of life or later [Atkinson et al., 1991; MMWR, 1989, 1994b, 1992; Tulchinsky et al., 1993]. In 1993 there was a record low number of measles cases in

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the United States, but in 1994 it began to rise primarily among young adults, apparently due to waning immunity [Ammari et al., 1993; Katz and Gellin, 1994; MMWR, 1994a, 1993].

In Israel, measles immunization was introduced in 1967 at age 9 months and later at age 12 months with the Schwartz strain of live attenuated vaccine. In 1973 the age was raised to 15 months, with the Edmonston vaccine strain. Coverage was 82% in 1970, 90% in 1975, 82% in 1983, and reached 88% in 1988–1989. The morbidity rate was reduced from 1,112/100,000 in 1950 to 0.57/100,000 in 1989 [Berger, 1991; Swartz, 1984; Tulchinsky, 1991; Tulchinsky et al., 1990]. An average morbidity rate of 22/100,000 was recorded in 1993–1994 [Epidemiological records, Israel, 1993–94]. However, outbreaks still occur every 5–7 years involving thousands of cases [Tulchinsky et al., 1990] among young babies, school-age children, and young adults. A second vaccine dose at age 6 years was introduced in 1990 as part of the routine childhood immunization schedule [Berger, 1991; Tulchinsky et al., 1993; Swartz, 1984; Tulchinsky, 1991; Tulchinsky et al., 1990].

In 1994, the age of the first dose was reduced to 12 month as a result of multiple cases among babies and the realization that babies born to immunized mothers lose their immunity early [Dagan et al., 1995; Epidemiological records, Israel, 1993–94; Katz and Gellin, 1994; Orenstein et al., 1994; Pabst et al., 1992].

Several studies examined the immune status and the response to revaccination of infants and young children but rarely of young adults [Bass et al., 1976; Orenstein et al., 1994; Markowitz et al., 1990, 1992; Murphy et al., 1984; Linnemann et al., 1982; Willy et al., 1994; Erdman et al., 1993]. An outbreak which occurred in the military and was terminated by a vaccination campaign provided us with the opportunity to investigate these parameters in a population of young adults.

In a previous report [Olsha et al., 1994] we compared the rates of fourfold or higher booster response among individuals with prevaccination HI titer of $<1:4$ and individuals with higher prevaccination HI titers. In this report we describe the use of the IgM and HI tests to analyze the true prevaccination immune status of the study population, the specific responses of various subgroups of this population, and the overall effect of the revaccination campaign.

SUBJECTS AND METHODS

Study Population

Two hundred eighty-nine soldiers (211 males and 78 females) aged 18–25 years were immunized against measles during a measles outbreak, using a live measles vaccine containing the Schwartz strain in order to prevent further spread of the disease. Eighty to 85% of these soldiers have been vaccinated as infants.

Serum Samples

Serum samples were collected from each individual in the study population immediately before immunization, and 14 and 28 days thereafter. Sera were stored at −20°C until use.

HI Test

Hemagglutination inhibition (HI) test was carried out according to standard procedures [Fuccillo and Sever, 1989] as follows: sera diluted 1:2 were treated with equal volumes of 25% (w/v) kaolin in PBS, incubated for 20 min at room temperature, and then spun down. The supernatant was then absorbed with Vervet monkey red blood cells (RBC) (50% suspension). Viral antigen was prepared from Vero cells infected with the Edmonston strain of measles virus by Tween-80 and ethyl-ether extractions. The hemagglutinin was titrated by adding 25 μl of 1% monkey RBC to 25 μl cell extract in V-shaped microtiter wells, shaking and incubating for 2 hr at 37°C. The highest dilution giving complete hemagglutination was determined to be 1 HA unit. To test the sera, serial twofold dilutions (1:4–1:512) in 25 μl of PBS were mixed with 25 μl antigen containing 4 HA units in V-shaped microtiter wells. The mixtures were incubated for 2 hr at room temperature; then 25 μl of 1% RBC suspension was added, and the samples were shaken and allowed to settle at room temperature for 1–2 hr. The titer of a serum sample was recorded as the highest serum dilution giving a complete inhibition of hemagglutination. Sera with perviously determined HI titers were used as controls and standards in each test.

ELISA for IgM

The Measles IgM Clark Laboratories Inc. diagnostic kit was used according to the manufacturer's instructions. The kit is based on a direct solid-phase enzyme immunoassay. The tested sera were preincubated with IgG absorbent solution before addition to the reaction plate. Following preincubation, sera were absorbed onto a 96-well plate precoated with measles antigen and incubated. Residual serum was washed away, and an enzyme-linked antiglobulin was added and incubated. The plate was washed again, the Substrate-chromogen was added, and the optical density was read. Negative control, low-positive calibrator, and high-positive calibrator were included for determination of cut-off value.

Statistical Analysis

The χ^3 and Mantel Haenszel χ^2 for trend tests were used, employing the SAS for PC V. 6.05 software. These tests were used to assess the statistical significance of the differences in the rates of HI and IgM seroconversion between subjects with low and high prevaccination titers.

RESULTS

The Population Immune Status Before and After the Vaccination

Antibody titers before vaccination and up to 28 days thereafter were determined by HI test in 289 vaccinees. The titers ranged from $<1:4$ to $\geq 1:512$. Following vaccination a clear shift from lower to higher titers was observed. Before the vaccination most subjects had antibody titers between 1:4 and 1:32, while after the

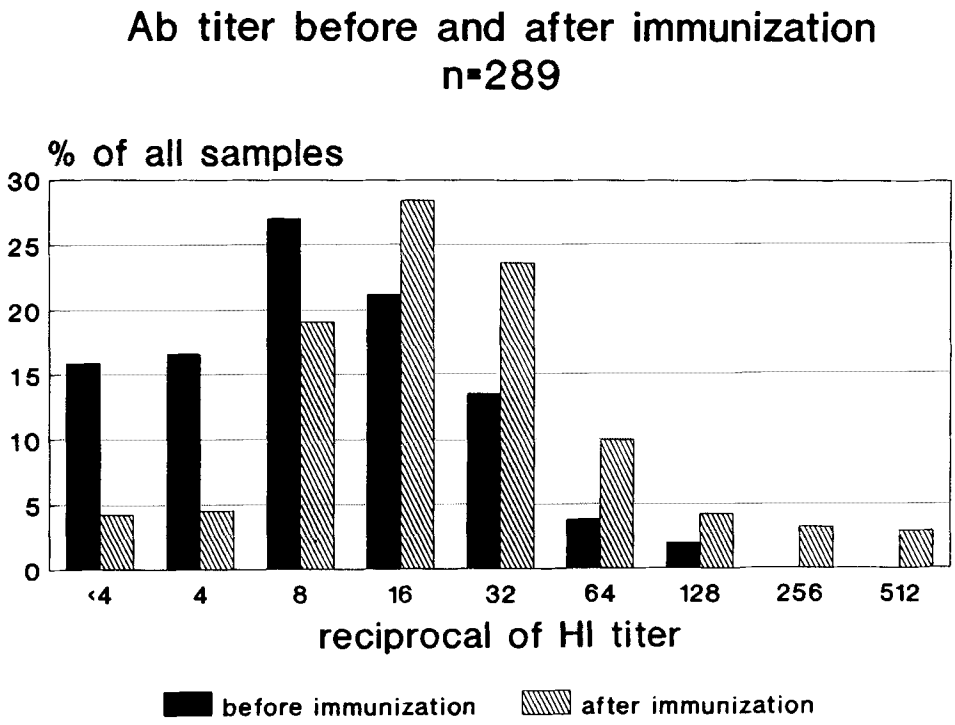

Fig. 1. The percentage of individuals with HI titers ranging from <1:4 to ≥1:512 before vaccination and 28 days afterward.

vaccination most of the subjects had antibody titers between 1:8 and 1:64 (Fig. 1). The percent of seronegatives (<1:4) dropped from 15.9% before vaccination to 3.5% 28 days afterward. The percent of borderline (1:4) dropped from 16.6% to 6.6% and the geometric mean titer (GMT) rose from 9.14 to 21.47.

Individual Response to the Vaccination

The association between the antibody titer before the vaccination and the extent of the booster response up to 28 days following the vaccination was examined. The results are summarized in Table I. The response to the vaccination was classified into one of three categories: an increase of fourfold and higher, a twofold increase, and no increase. A change from a prevaccination titer of <1:4 to a postvaccination titer of ≥1:8 was considered a fourfold or higher increase, while a change from <1:4 to 1:4 was considered a twofold increase.

The data obtained show an inverted correlation between the prevaccination titer and the presence or absence of booster response. The percent of subjects with a less than fourfold increase rose steadily with higher prevaccination titers and reached 96.4% among individuals with ≥1:32 HI titer (Mantel Haenszel χ^2 for trend $P < 0.001$). Overall, 159 subjects (55.0%) showed no increase. Interestingly, in 30 out of 130 individuals (23%) with greater than twofold response the increase in HI titer was observed only in the samples taken 28 days after the vaccination and not before.

IgM Response to Vaccination

Two hundred twenty-five of the vaccinees (including all the prevaccination negative and borderline subjects) were tested for IgM response at 14 and 28 days following the vaccination. The results, shown in Table II, demonstrate the association with prevaccination titer. Among the 20 vaccinees who developed IgM antibodies, 12 had negative prevaccination HI titer, while three had borderline (1:4) and five had positive HI titers before the vaccination (χ^2, $P < .001$). Seven out of eight prevaccination-positive individuals had IgM titers already at 14 days following vaccination, while among the prevaccination-negative individuals only five out of 12 had IgM antibodies at day 14.

Response of Prevaccination-Negative Individuals

In order to examine whether the prevaccination sera with HI titer less than 1:4 reflected true cases with no previous or residual immunity to the virus, we compared the results of the enzyme-linked immunosorbent assay (ELISA) IgM test performed on serum samples taken at 14 and 28 days following the vaccination to the results of the postvaccination HI test which can demonstrate seroconversion. The results are summarized in Table III. HI antibody titer of 1:8 or higher at 14 or 28 days following the vaccination was regarded as seroconversion.

Forty-six vaccinees had negative prevaccination HI

TABLE I. Association Between Prevaccination Titer and Antibody Increase 28 Days After Vaccination

Prevaccination titer	No. of samples	≥ × 4 increase[a,*]		× 2 increase[b]		No increase	
		No.	%	No.	%	No.	%
<1:4	46	28	60.9	8	17.4	10	21.7
1:4	48	18	37.5	19	39.6	11	22.9
1:8	78	15	19.2	24	30.8	39	50.0
1:16	61	6	9.8	8	13.1	47	77.0
1:32	39	1	2.6	0	0	38	97.4
1:64	11	0	0	2	18.2	9	81.8
1:128	6	1	16.7	0	0	5	83.3
All	289	69	23.9	61	21.1	159	55.0

[a]A change from <1:4 to ≥1:8 was considered a fourfold or higher increase in this group.
[b]A change from <1:4 to 1:4 was considered a twofold increase in this group.
*$P < .001$ by Mantel-Haenszel χ^3 for trend.

TABLE II. IgM Response to Vaccination Among 225 Subjects

Prevaccination titer	First IgM response		Total IgM response
	14 days	28 days	
<1:4	5	7	12
1:4	3	0	3
1:8	1	0	1
1:16	3	0	3
1:32	0	1	1
≥1:64	0	0	0
Total	12	8	20

titer. Out of the 46, 12 (26%) developed IgM antibodies either at 14 or at 28 days following the vaccination and seroconverted, indicating a primary response. Sixteen of the 46 (35%) did not develop IgM but seroconverted, indicating a secondary booster response. Eighteen of the 46 negatives (39%) did not develop IgM and also did not seroconvert, suggesting a primary vaccine failure.

DISCUSSION

The best immunization schedule that would lead to elimination of measles cases in the world has not yet been determined. More than 20 years of massive immunization with one dose only at age 9–15 months created populations of young adults with low immunity. The immunization campaign in several army units following an outbreak in 1991 allowed us to collect more information regarding this topic.

Our study population represents the general Jewish population in Israel. The HI tests, one of the two most reliable tests for determination of immunity to measles, was used for the routine serological survey. It is usually in good correlation with the neutralization test which is more sensitive and considered the "Gold Standard," but is very cumbersome and was therefore not used. Both tests are considered to reflect the true immune status and the level of protection against measles [Black, 1989; Fulginity and Kempe, 1965; Lerman et al., 1993; Chen et al., 1990]. Our yearly serological surveys of Israeli school children aged 10–16 years [Duvdevani et al., 1989–1993] showed that 10–15% are not immune (HI <1:4) and an additional 5% have borderline immunity. The current study examined a population of young soldiers aged 18–25 years using the same HI test. Fifteen to 20% of these soldiers might not have been immunized in their childhood because of the low vaccine coverage in the early years of immunization [Tulchinsky, 1991].

The results of screening showed that the percent of non-immune was 15.9% and the rate of borderline immunity was 16.6%. This data demonstrate some decrease in immunity over time. However, using the IgM test, we could show that only 26% of the HI-negative individuals (5.3% of the entire study group tested for IgM) were "true negatives," since they developed IgM antibodies. Thirty-five percent of the HI negative individuals may have had residual immunity since they did not develop IgM antibodies, yet seroconverted [Erdman et al., 1993; Linnemann et al., 1973]. Thirty-nine percent of the negative individuals did not respond at all, suggesting a specific immune deficiency or a failure of the vaccine. This group, which is 8% of the study population tested for IgM, may represent the normal rate of primary vaccine failure but, alternatively, may include subjects with very low antibody titers undetectable by the HI test, which prevented them from responding to revaccination, as was shown for younger children and babies [Linnemann et al., 1982; Murphy et al., 1984; Bass et al., 1976].

The response to revaccination among positive individuals was related to their prevaccination titer. The weak response of individuals with HI titer of 1:16 and higher confirmed previous observations that current immunity can prevent further response [Black et al., 1984]. Thus, revaccination can be principally counted on to immunize the true primary vaccine failures and to booster the borderline cases, while the benefit to others may be limited.

We encountered eight individuals who had HI antibodies prior to the vaccination but developed IgM titer afterwards, seven of them less than 14 days following the vaccination. It is assumed that these individuals were infected with the circulating wild-type virus prior to the vaccination [Erdman et al., 1993] but did not develop clinical symptoms due to their residual immunity.

TABLE III. Response to Vaccination of Individuals With Negative (<1:4) Prevaccination HI Titer

	IgM^+/seroconversion[a]			IgM^-/seroconversion[a]			IgM^-/no seroconversion:	
	14d	28d	Total	14d	28d	Total	total	Total
No.	5	7	12	10	6	16	18	46
%	11	15	26	24	22	35	39	100

[a]A change from a titer of <1:4 to a titer of ≥1:8 by the HI test either on the 14th day or on the 28th day following immunization.

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